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Effect of Cold Acclimation on Bulk Tissue Electrical Impedance

I. MEASUREMENTS WITH BIRDSFOOT TREFOIL AT SUBFREEZING TEMPERATURES

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DARRYL G. STOUT
Agriculture Canada, Range Research Station, Kamloops, B.C., Canada V2B 8A9

ABSTRACT

The resistive and reactive components of electrical impedance were measured for birdsfoot trefoil (*Lotus corniculatus* L.) stems at freezing temperatures to −8°C. As temperature decreased the specific resistance at frequencies between 49 hertz and 1.11 megahertz of stems from cold acclimated plants increased more rapidly than from nonacclimated plants. This temperature dependence of specific resistance could be characterized by an Arrhenius activation energy; cold acclimated stems had a larger Arrhenius activation energy than nonacclimated stems. The low frequency resistance is believed to characterize the extracellular region of the stems and the high frequency resistance is believed to characterize the intracellular region of the stems. Cold acclimation increased the intracellular but not the extracellular resistance at nonfreezing temperatures. Cold acclimated stems were not injured by freezing to −8°C and thawing, but nonacclimated stems were injured by freezing to temperatures between −2.2 and −5.6°C and thawing. Injury to nonacclimated stems at freezing temperatures below −2.2°C was indicated by a decrease in the ratio of resistance at 49 Hz to that at 1.11 megahertz.

Large voltages develop at the ice water interface when dilute aqueous solutions are frozen (27). Freeze-induced electrical transients also occur when solutions containing protoplasts are frozen, but it is not known if these electrical transients can develop across the cell membranes and injure them (19, 20). Protoplasts from ACCL[1] rye (*Secale cereale* L.) plants survive stronger electric fields than protoplasts from nonacclimated plants (20). These recent results indicate that the electrical characteristics of plants at freezing temperatures may be important to plant survival at freezing temperatures.

In the past, measurements of electrical impedance at room temperature have been used to detect freeze-thaw injury to plants (5, 7, 8, 26). Freezing injury decreases the impedance owing to membrane damage, and the decrease is larger at lower frequencies. Thus, the effect of freezing injury is typically evaluated using either a low frequency impedance, or an impedance ratio where a low frequency impedance is divided by a high frequency impedance. Greenham and Daday (8) used 1000 Hz as a low frequency and 1.0 MHz as a high frequency. Hayden *et al.* (10) recommended using 60 Hz for the low frequency impedance measurement.

Greenham (7) measured plant tissue electrical impedance at freezing temperatures. He froze alfalfa (*Medicago sativa* L.) to −6°C and measured the electrical resistance for 6 h; during this time, the resistance decreased indicating that membrane permeability increased and membrane injury occurred while plants were in a frozen state.

As temperature decreases, electrical impedance gradually increases at nonfreezing temperatures, and then increases rapidly at freezing temperatures (1, 3, 23). For example, Hayden et al. (10) measured the electrical conductance (1/resistance) of alfalfa over the temperature range 5 to −6°C. E* was then calculated from Arrhenius plots of log (conductance) *versus* T^{-1}. For unfrozen tissue, E* ranged between 15.5 and 42.7 kJ mol^{-1} K^{-1}. For frozen tissue, two categories of E* occurred: 84 to 251 kJ mol^{-1} K^{-1} and greater than 335 kJ mol^{-1} K^{-1}. In contrast to the

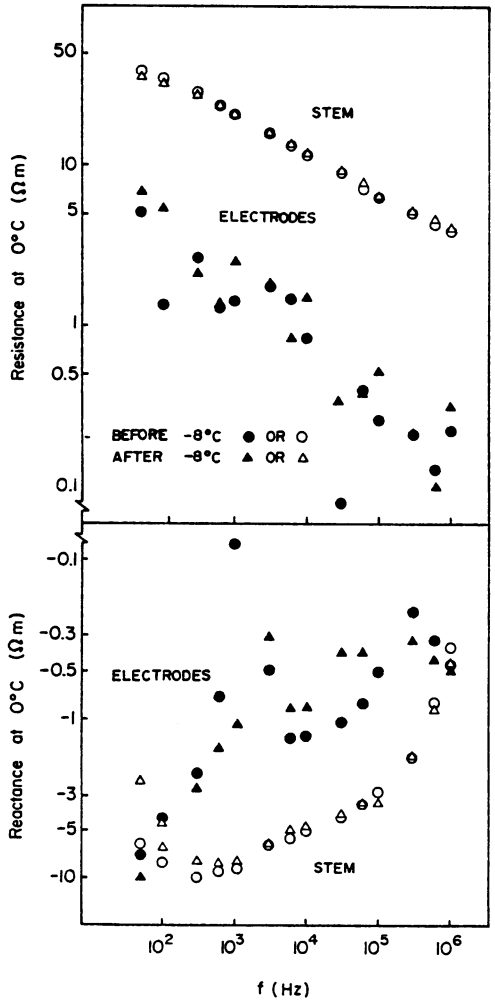

FIG. 1. Effect of freezing to −8°C and thawing on the frequency dependence of stem and electrode specific resistance and specific reactance for ACCL tissue. Values are means from 3 stems.

[1] Abbreviations: ACCL, cold acclimated; E*, Arrhenius activation energy; τ, time constant; NA, nonacclimated.

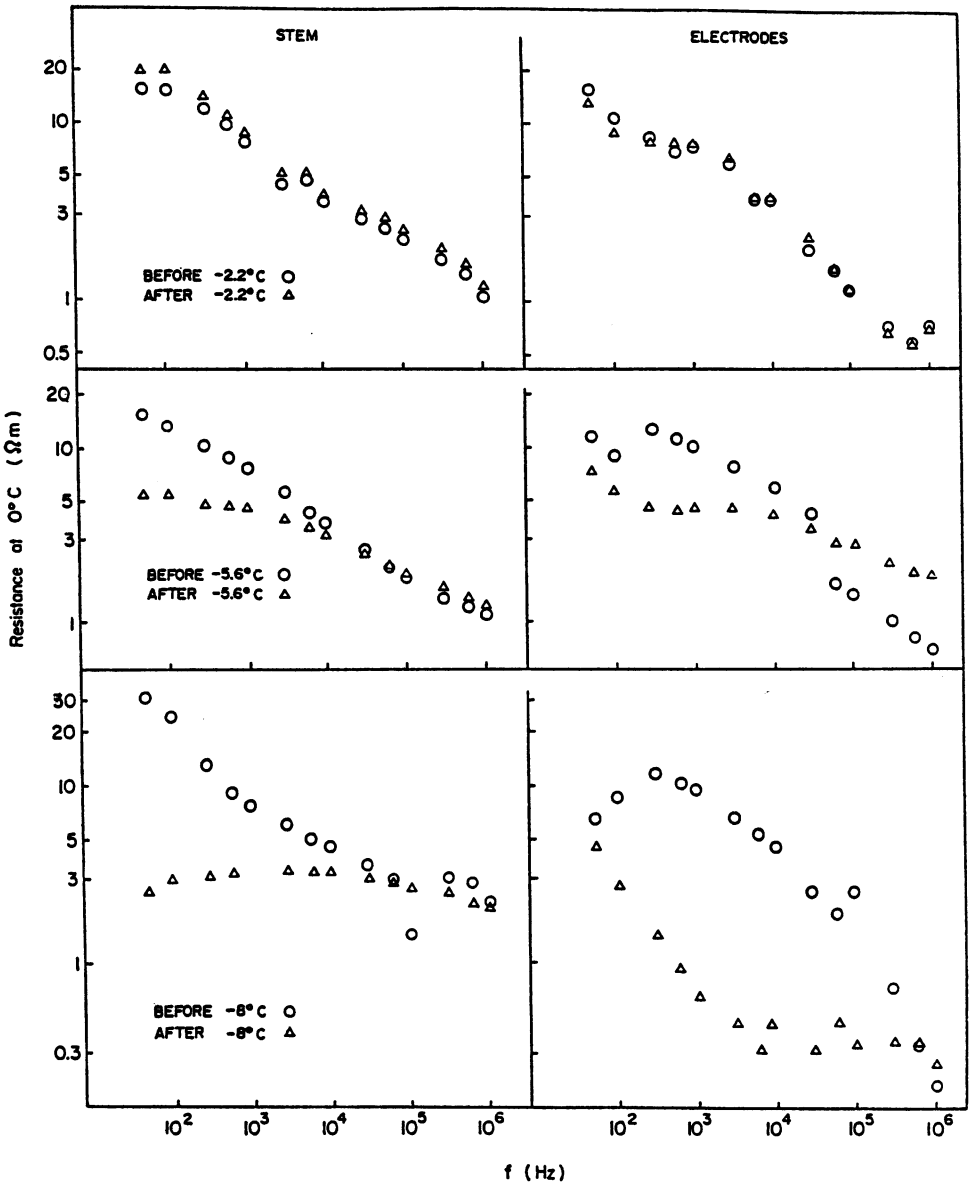

FIG. 2. Effect of freezing to −2.2, −5.6, or −8°C and thawing on the frequency dependence of stem and electrode specific resistance for NA tissue. Values are means from 2 stems.

linear relationship between log (conductance) and T^{-1} reported by Hayden *et al.* (10), other researchers (1, 3, 23) reported a linear relationship between log (resistance) and T. Walton (23) studied the effect of cold acclimation on the temperature dependence of tissue impedance and reported that the temperature dependence was similar for ACCL and NA tissue at freezing temperatures but was different at nonfreezing temperatures.

The first objective of the work reported here was to determine the effect of freezing and of freeze-thawing on the electrical characteristics of birdsfoot trefoil (*Lotus corniculatus* L.) stems. The second objective was to determine if cold acclimation alters the effect of freezing and freeze-thawing on the electrical characteristics.

MATERIALS AND METHODS

Growth and Cold Acclimation of Plants. Birdsfoot trefoil seeds (cv Leo) were planted into 6 dm^3 plastic pots containing a soil mix consisting of two parts loam, two parts sand, and one part peat. Following germination, six plants per pot were grown for 9 months in a growth-chamber with a 12 h photoperiod, a PAR of 133 μmol m^{-2} s^{-1} provided by fluorescent (Vita-Lite, Duro-Test Corp., NJ) and incandescent lamps, a day temperature of 20°C, and a night temperature of 18°C. At 18 and 34 weeks, plant top growth was removed leaving a 2 cm stubble. When the plants were 40 weeks old, one-half of the pots were transferred to a second growth-chamber for cold acclimation; 8 h photoperiod, PAR of 300 μmol m^{-2} s^{-1}, day temperature 8°C and night temperature 2°C. Electrical measurements were begun following 7 weeks of cold acclimation and continued for 8 d. The temperature dependence at above freezing temperatures was done using 69 week old NA plants that had been kept in a vegetative stage of development by occasional clipping to a 2 cm stubble.

Electrical Measurements. A complete consideration of plant impedance requires a consideration of both the resistance and reactance. Stout *et al.* (21) recently outlined a procedure that estimates both the real and complex parts of the impedance (*i.e.* the resistive and reactive parts) given measured values of voltage and phase angle. This method separates the plant and electrode impedances. In reality, the electrode impedance calculated is composed of the actual electrode impedance and the electrode-stem contact impedance. The apparatus and methods used in this study were identical to those described by Stout *et al.* (21).

For electrical measurements, a stem was removed from a plant, the leaves were removed from the stem, the stem diameter was measured with a micrometer, the stem was cut in half, and one-

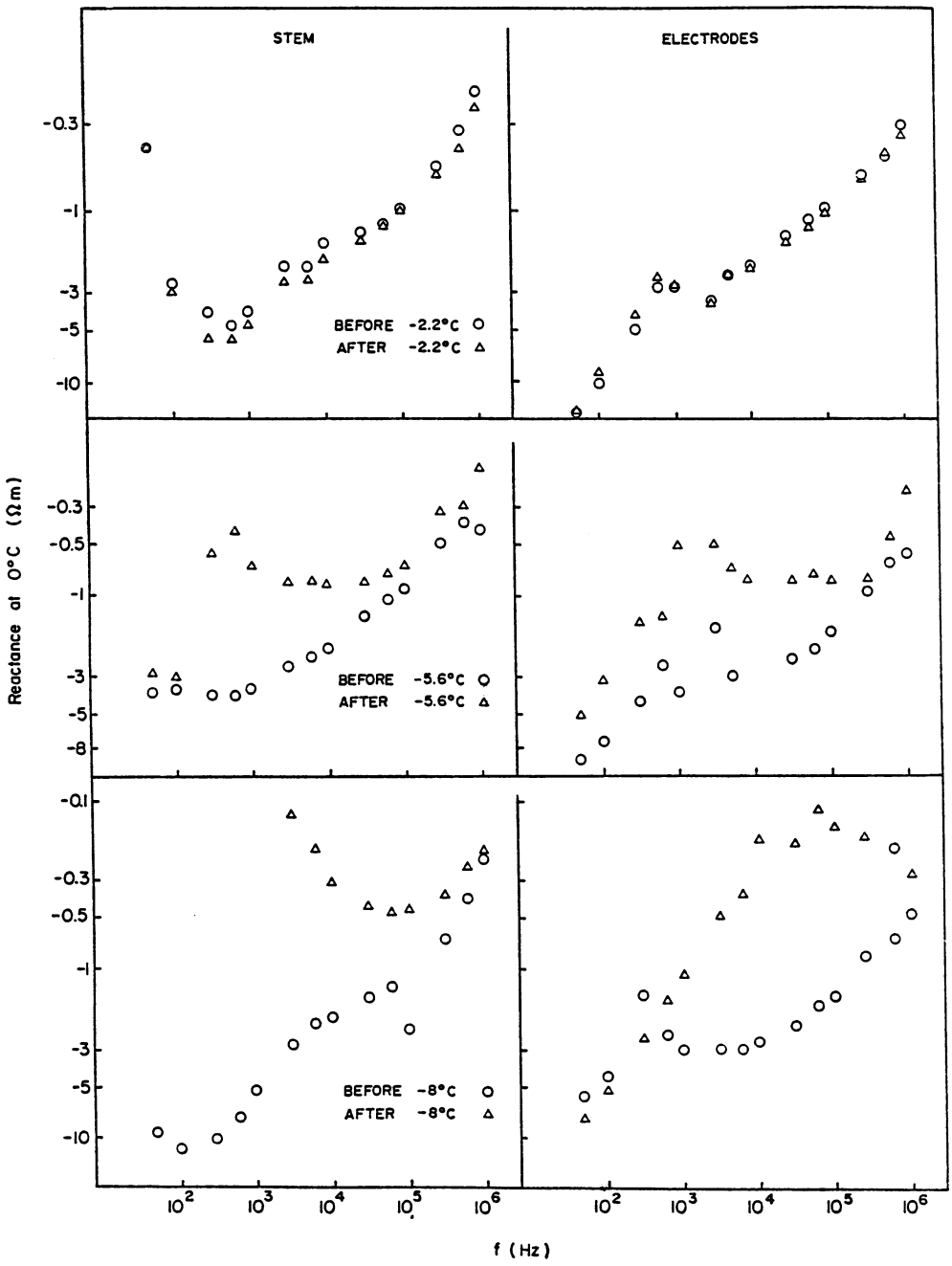

FIG. 3. Effect of freezing th −2.2, −5.6, or −8°C and thawing on the frequency dependence of stem and electrode specific reactance for NA tissue. Values are means from 2 stems.

half of each stem half was coated with vaseline grease (14) and wrapped in Saran Wrap to decrease dehydration and prevent conduction through moisture on the outside of the stem. The vaseline coated part of the stem was impaled onto the electrode and the noncoated part was used to accommodate initiation of freezing. One stem half was impaled on the tips of a pair of electrodes 9.5 mm apart and the other stem half was impaled on the tips of a pair of electrodes 29 mm apart. Current flowed through the stem longitudinally. The diameter of the straight pins at the surface of a stem was approximately 0.38 mm. The end (about 1 cm) of each stem half that was not coated with grease was wrapped in a wet Kimwipe tissue. Stems impaled on electrodes were then placed in a domestic freezer equipped with a heater and a temperature controller, at 0°C. The temperature was then lowered to −2.2°C. After 0.5 h ice crystals from a freezer were placed on the wet Kimwipe tissue to initiate ice formation. Electrical measurements were begun following a further 0.5 h temperature equilibration period. The temperature was then either lowered or raised (specific temperature protocols are shown in the figures) and a 0.5 h temperature equilibration was allowed before electrical measurements were begun. Owing to a low signal to noise ratio, it was not possible to make measurements at temperatures below −8°C.

Voltages and phase angles were measured at 14 frequencies: 49; 101; 305; 598; 1010; 3030; 5940; 10,000; 30,300; 59,600; 100,000; 304,000; 667,000; and 1,110,000 Hz. It took about 10 min to carry out measurements for a single stem at 14 frequencies and two electrode spacings.

Statistical Methods. For each specimen and frequency, the relationship between the resistance or reactance and the distance between the electrodes was fitted by linear regression. The slopes became estimates for the plant tissue and the intercepts became estimates for the electrodes (Eq. 7 [21]). Estimates of resistance and reactance were multiplied by stem cross-sectional area to

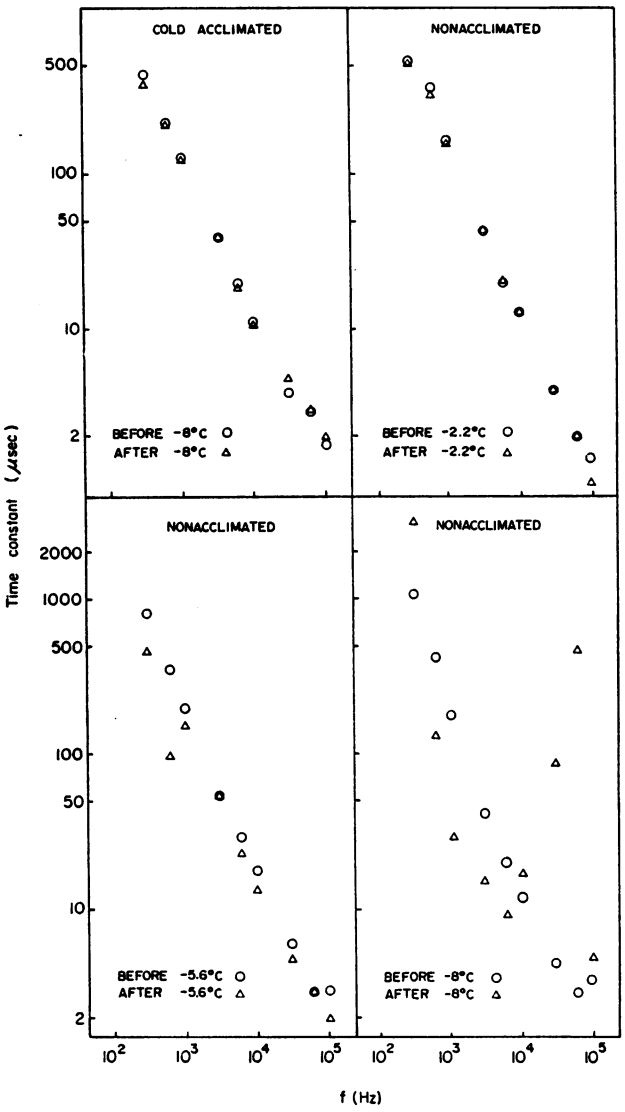

FIG. 4. Effect of freezing and thawing on the frequency dependence of the time constant of ACCL and NA tissue. Values are means from 3 ACCL stems and 2 NA stems.

account for variation in stem size. Means and standard errors were calculated and are reported in the figures. To simplify presentation, data are not given for all frequencies.

RESULTS

Effect of Freeze-Thawing on Resistance, Reactance, and Time Constant. Both resistance and reactance of stems and electrodes for cold acclimated plants were unaffected by freezing to −8°C and thawing to 0°C (Fig. 1). Therefore freezing to −8°C did not injure ACCL stems. Similarly, freezing NA stems to −2.2°C and thawing did not alter the resistance or reactance of stems or electrodes (Figs. 2 and 3). Freezing NA stems to −5.6°C or −8°C and thawing to 0°C altered the resistance and reactance of both stems and electrodes. Therefore NA stems were injured at a freezing temperature between −2.2 and −5.6°C. The freeze to −5.6°C and thaw decreased stem resistance at frequencies between 49 and 3030 Hz (Fig. 2); the freeze to −8°C and thaw decreased stem resistance at these frequencies even more. An effect of freezing to −5.6 and −8.0°C and thawing was also observed for resistance of electrodes (Fig. 2) and for reactance of NA stems and electrodes (Fig. 3).

The magnitude for electrode resistance or reactance at 0°C was about 10 to 20% of the magnitude for ACCL stem resistance or reactance (Fig. 1). In contrast, at 0°C the electrode resistance for NA stems was about 50 to 80% of that for stems (Fig. 2). For NA stems, the electrode reactance was about the same magnitude as the stem reactance (Fig. 3).

The relative magnitude of reactance to resistance can be demonstrated by comparing values at one frequency. For example, at 3030 Hz the magnitude of the reactance was 39% of the resistance for ACCL stems (Fig. 1). For NA stems the value was 47% (Figs. 2 and 3). Thus, the ratio of reactance to resistance was similar for both ACCL and NA stems. Results for electrodes, however, were different for the two different types of tissue. For ACCL stems the reactance of electrodes at 3030 Hz was 22% of the resistance of electrodes. For NA stems electrode reactance was 57% of electrode resistance.

At each frequency τ was calculated according to Stout *et al.* (21) for ACCL stems frozen to 8°C and thawed to 0°C, and for NA stems frozen to −2.2°C and thawed to 0°C, τ was not affected by freeze-thawing (Fig. 4). Following freezing of NA stems to −5.6 or −8°C, τ was different than it had been before freezing.

Effect of Freezing on Resistance, Reactance, and Time Constant. As the temperature was lowered to −8°C, the resistance of ACCL stems increased more rapidly than the resistance of NA stems (Figs. 5–7). For example, at 49 Hz and 0°C the resistance of ACCL stems was 1.75 times the resistance for NA stems, at −8°C this ratio increased to 5.34 (Fig. 5). This effect of cold acclimation on the temperature dependence of resistance is characterized by a greater activation energy for ACCL stems (Table I). At higher frequencies, such as 1.11 MHz, a similar trend was observed (Fig. 7) but the difference in activation energies for NA and ACCL stems was smaller (Table I).

Reactance of ACCL stems had a greater magnitude than reactance of NA stems (Figs. 5–7). But at the highest frequency, 1.11 MHz, the reactance of both NA and ACCL stems was close to zero; therefore, at such high frequencies, nearly all of the impedance is resistance.

At the lower frequencies, ACCL stems had a greater electrode resistance than NA stems at the lower freezing temperatures (Fig. 5). However, at higher freezing temperatures or at higher frequencies both types of stems had similar resistances (Fig. 5–7). Reactance showed a similar relationship to resistance for both ACCL and NA stems (Figs. 5–7).

At above freezing temperatures, the activation energy for resistance and reactance of NA stems averaged 18.4 kJ mol^{-1} K^{-1} (Fig. 8; Table II). The activation energy for the time constant averaged 13.4 kJ mol^{-1} K^{-1}, but the correlation between ln τ and T^{-1} was not statistically significant. At freezing temperatures the activation energies were much higher (Table I) than at nonfreezing temperatures. Since the correlation between resistance, reactance, or time constant and temperature was generally not statistically significant for NA stems, it is not clear that the temperature dependence can be characterized by an activation energy. However, the correlation was normally highly significant for ACCL stems (Table I).

At freezing temperatures, the resistance of the electrodes is approximately equal to the resistance of ACCL stems but exceeds the resistance of NA stems (Figs. 5–7). A similar relationship holds for reactance at low frequencies (Figs. 5 and 6); however, at high frequencies, electrode reactance exceeds plant reactance for both ACCL and NA tissue (Fig. 7).

Effect of Freeze-Thawing and Freezing on the Resistance Ratio. The ratio of resistance at 49 Hz to resistance at 1.11 MHz for ACCL stems was not significantly affected by freezing to −8°C and thawing (Fig. 9). Similarly the resistance ratio of NA stems was not changed significantly by freezing to −2.2°C and thawing. However, freezing NA stems to −5.6°C or −8°C and

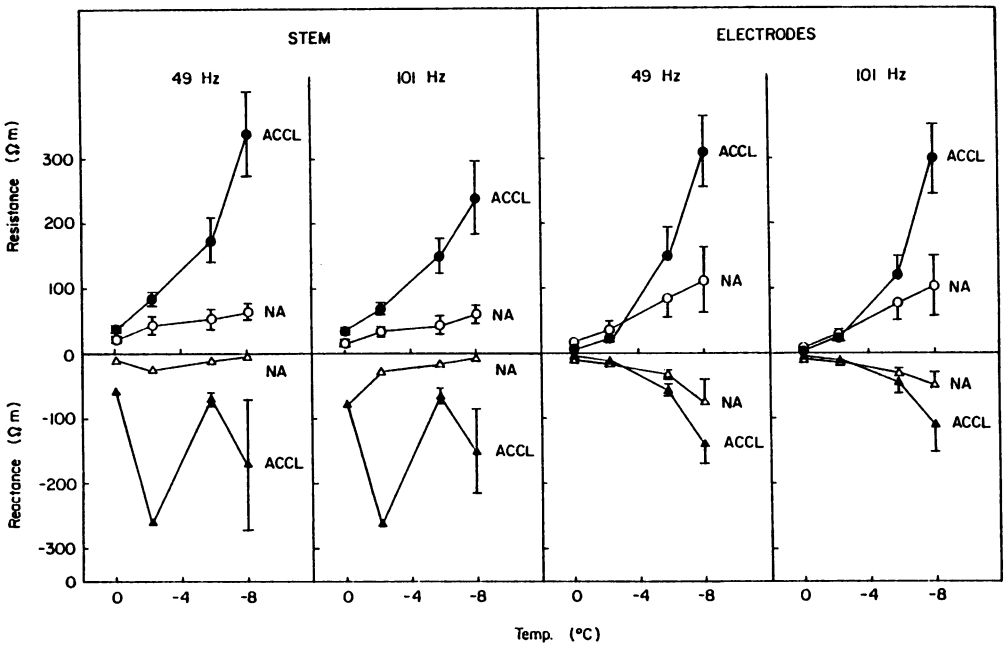

FIG. 5. Effect of cold acclimation on specific resistance and specific reactance of stems and electrodes at low frequencies. Values are $\bar{x} \pm$ SE. Where error bars are not shown, the error was smaller than the symbol used for graphing. For ACCL tissue $n = 3$. For nonacclimated tissue $n = 6$ at 0 and −2.2°C, $n = 4$ at −5.6°C, and $n = 2$ at −8°C.

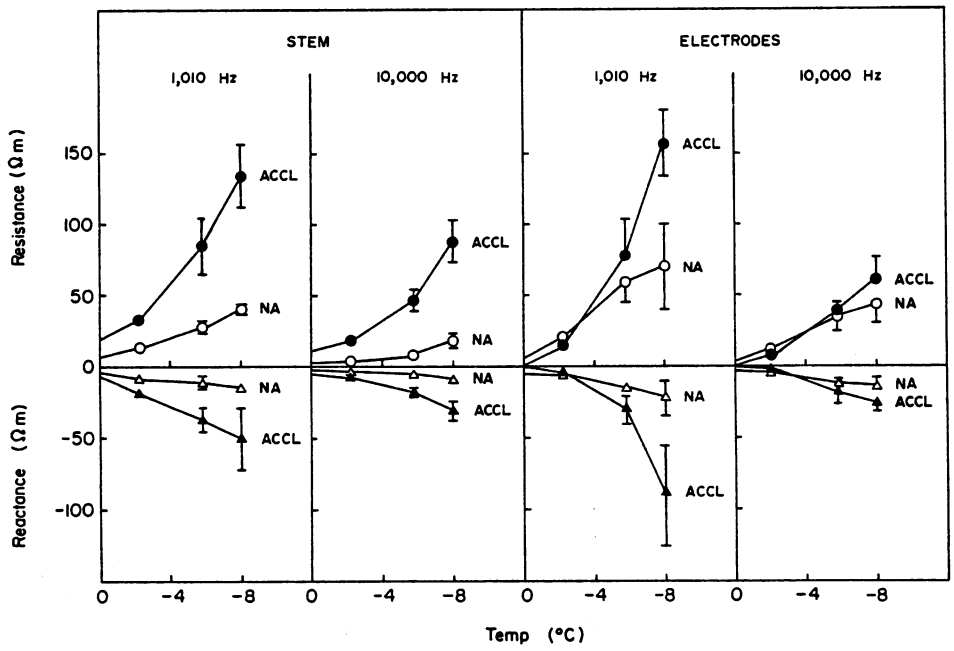

FIG. 6. Effect of cold acclimation on specific resistance and specific reactance of stems and electrodes at intermediate frequencies. Values are $\bar{x} \pm$ SE. Where error bars are not shown, the error was smaller than the symbol used for graphing. For ACCL tissue $n = 3$. For NA tissue $n = 6$ at 0 and −2.2°C, $n = 4$ at −5.6°C, and $n = 2$ at −8°C.

thawing, decreased the resistance ratio; freezing to −8°C and thawing decreased the resistance ratio more than freezing to −5.6°C and thawing.

The resistance ratio for ACCL stems remained relatively constant at temperatures between 0 and −8°C (Fig. 10). In contrast, the resistance ratio for NA stems decreased with decreasing temperature.

DISCUSSION

Both the electrode impedance and the reactive component of impedance need to be accounted for when measuring plant tissue impedance. In contrast to an earlier report (6), this study found that the impedance of bare metal electrodes is significant. As well, electrode impedance changes with freeze-injury (Fig. 3), and with cold acclimation, temperature and frequency (Figs. 5–7). Therefore, when comparing plants in different physiological states, it cannot be assumed that a total impedance change simply indicates a change in plant impedance. Some studies have assumed that plant impedance is simply resistive (9, 23) but this study has revealed that the reactive component can also be substantial (Figs. 5–7).

Hayden *et al.* (10) proposed a simple and useful model for plant electrical impedance. With this model, low frequency impedance characterizes the extracellular pathway and high frequency impedance characterizes the intracellular pathway. Stout *et al.* (21) found that the resistance of alfalfa stems measured at 15 Hz was about 1.1 times the resistance measured at 49 Hz. Therefore, measurement of impedance at 49 Hz is not strictly a measure of the extracellular resistance. This is especially true for ACCL tissue where there is also a substantial reactance at 49 Hz (Fig. 5). Nevertheless, because the phase angle is more difficult to measure at 15 Hz than at 49 Hz, the lowest frequency used in this study was 49 Hz and it is assumed that the impedance at this frequency largely characterizes the extracellular environment. The assumption that high frequency (*e.g.* 1.11 MHz)

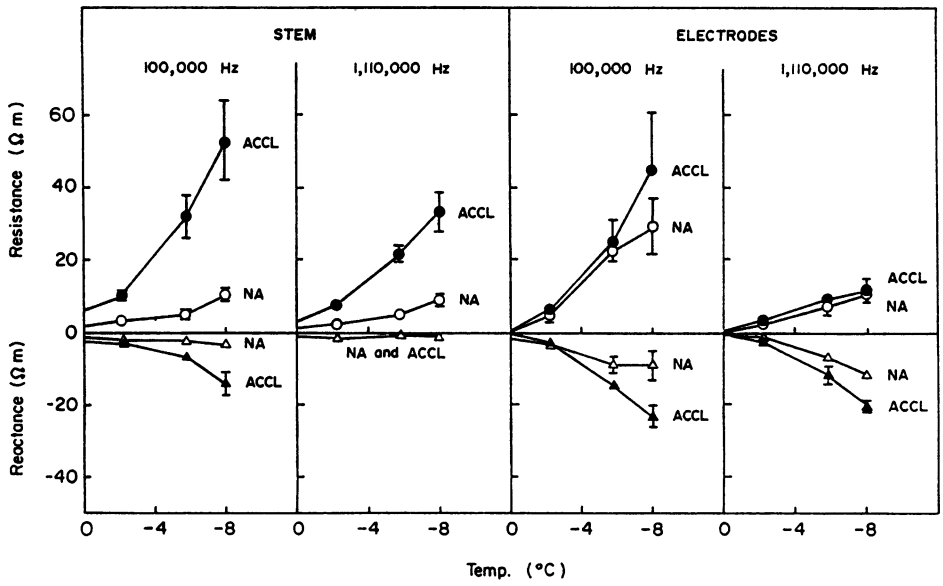

FIG. 7. Effect of cold acclimation on specific resistance and specific reactance of stems and electrodes at high frequencies. Values are $\bar{x} \pm$ SE. Where error bars are not shown, the erros was smaller than the symbol used for graphing. For ACCL tissue $n = 3$. For NA tissue $n = 6$ at 0 and −2.2°C, $n = 4$ at −5.6°C, and $n = 2$ at −8°C.

Table I. *Effect of Cold Acclimation on Activation Energies (E*) for Stems between 0°C and −8°C*

Sample size was 12 for cold acclimated and 18 for nonacclimated.

Measure	Frequency	Nonacclimated		Cold acclimated	
		E*[a]	r[b]	E*	r
	Hz	*kJ mol⁻¹ K⁻¹*			
Resistance	49	82	0.383 NS	153	0.941**
	101	91	0.471*	134	0.914**
	1,010	136	0.671**	138	0.937**
	10,000	97	0.383 NS	148	0.965**
	100,000	121	0.704**	160	0.954**
	1,110,000	147	0.845**	166	0.973**
Reactance	49	−18	0.277 NS	209	0.823**
	101	−10	0.264 NS	202	0.899**
	1,010	82	0.405 NS	113	0.718**
	10,000	121	0.792**	131	0.916**
	100,000	81	0.446 NS	116	0.913**
	1,110,000	−90	0.257 NS	133	0.471 NS
Time constant	589	−62	0.285 NS	−54	0.778**
	3,030	−57	0.095 NS	−39	0.732**
	10,000	−1	0.245 NS	−27	0.668*

[a] E* was estimated from a linear least square fit of the data to an Arrhenius equation. [b] Correlation coefficient: * indicates significance at $P \leq 0.05$; ** indicates significance at $P \leq 0.01$; and NS indicates not significantly different from the Null hypothesis: $r = 0$.

Table II. *Activation Energies for NA Stems over the Temperature Range 3.9 to 24.4°C*

Measure	Frequency	E*[a]	r[b]
	Hz	*kJ mol⁻¹ K⁻¹*	
Resistance	49	18.0	0.794*
	101	17.6	0.875**
	1,010	14.2	0.879**
	10,000	18.0	0.971**
	100,000	14.6	0.963**
	1,110,00	13.4	0.976**
Reactance	49	28.5	0.796**
	101	26.8	0.899**
	1010	16.7	0.854**
	10,000	19.7	0.959**
	100,000	14.2	0.828**
	1,110,00	18.8	0.529 NS
Time constant	598	5.9	0.430 NS
	3,030	18.0	0.488 NS
	10,000	16.7	0.550 NS

[a] Value estimated from a linear least square fit of the 8 data points to an Arrhenius equation. [b] Correlation coefficient: * indicates significance at $P \leq 0.05$; ** indicates significance at $P \leq 0.01$; and NS indicates not significantly different from the Null hypothesis: $r = 0$.

impedance characterizes the intracellular environment is valid since this impedance is not significantly different from the average impedance measured for dead stems at all frequencies (21).

Cold acclimation did not have a significant effect on resistance at 49 Hz when measured at 0°C (Fig. 5). Several studies have reported that low frequency resistance increased during cold acclimation (4, 9, 25, 26). The difference between the results shown in this study and published results could be related to the fact that reactance and electrode resistance were accounted for in the current study, or different species were used, or both. At freezing temperatures, however, resistance of ACCL stems differed dramatically from resistance of NA stems: ACCL stems had a larger activation energy than NA stems (Table I). Clearly, the extracellular environment of ACCL stems is different than the extracellular environment of NA stems during freezing. This observation could have implications on generation of electrical field transients during extracellular freezing, and on whether or not these electrical field transients can develop across cell membranes.

The high frequency resistance of ACCL stems is greater than the high frequency resistance of NA stems at both nonfreezing and freezing temperatures (Fig. 7). Steponkus *et al.* (19) reported that ACCL rye protoplasts had a higher K^+ content than NA protoplasts. Stout *et al.* (21) showed that K^+ content of birdsfoot trefoil stems remained constant during cold acclimation but, because the water content decreased, the concentration of K^+ increased. Since K^+ is the major cation in plant tissue (16) it would be expected that increased K^+ concentration would decrease rather than increase the resistance. However, sugars are known to increase during cold acclimation of birdsfoot trefoil (11); and sucrose increases electrical resistance (24). Therefore the increase in resistance of the intracellular solution likely reflects an increase in viscosity owing to sugars and other cellular constituents which increase during cold acclimation (18), which

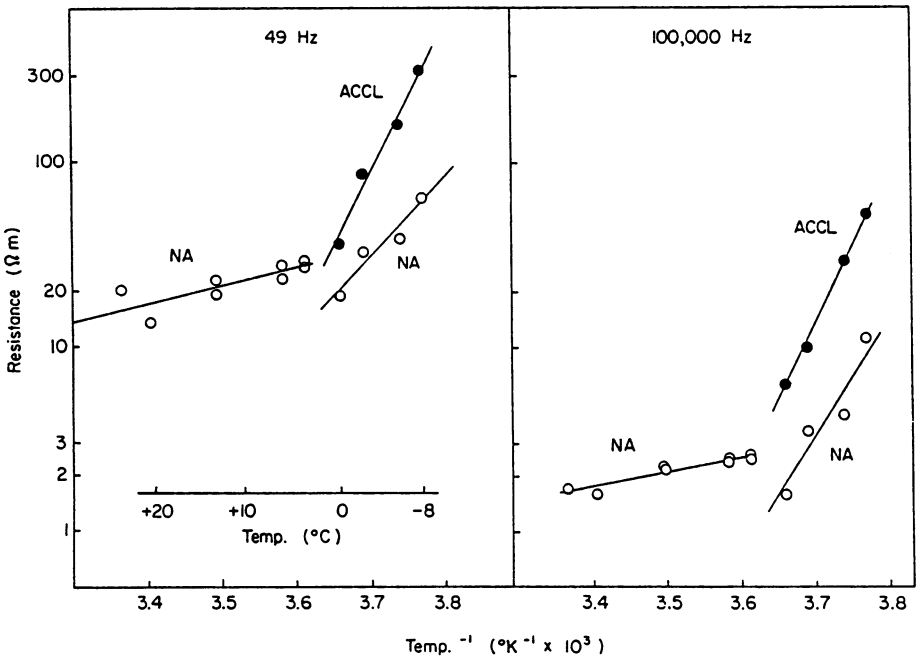

FIG. 8. Arrhenius plots for specific resistance of ACCL and NA stems. Above 0°C, values are for $n = 1$. Below 0°C, values are means. For ACCL tissue $n = 3$. for NA tissue $n = 6$ at 0°C and −2.2°C, $n = 4$ at −5.6°C, and $n = 2$ at −8°C.

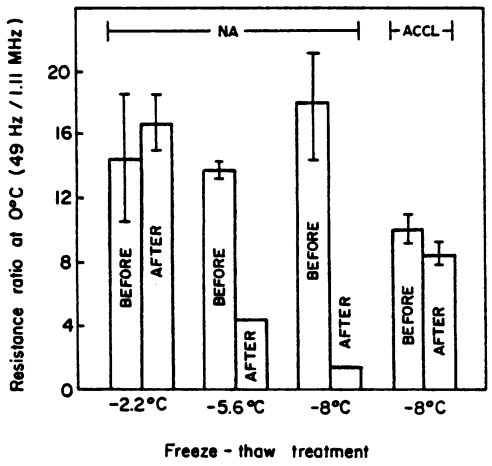

FIG. 9. Effect of freeze-thaw treatments on low-high frequency resistance ratio. Values are means ± SE. Where error bars are not shown, the error was smaller than the symbol used for graphing. For ACCL tissue $n = 3$ and for NA tissue $n = 2$.

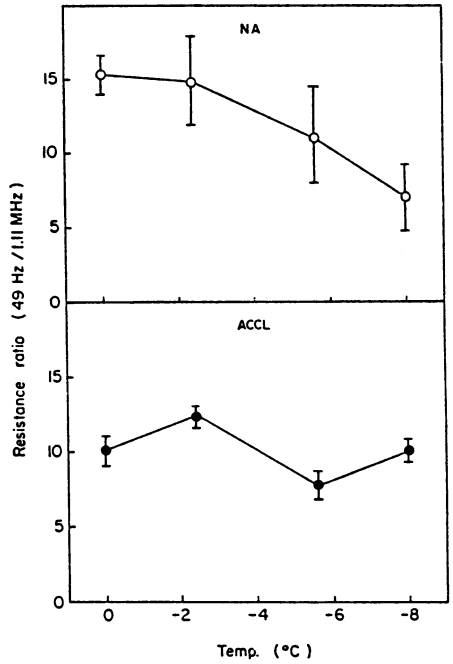

FIG. 10. Effect of cold acclimation on the low-high frequency resistance ratio at freezing temperatures. Values are means ± SE. For ACCL tissue $n = 3$ and for NA tissue $n = 6$ at 0°C and −2.2°C, $n = 4$ at −5.6°C, and $n = 2$ at −8°C.

more than offset the decreased resistance caused by increasing electrolyte concentration. Olien (13) concluded that movement of electrolytes in cell walls at low temperatures reflected the viscosity of the extracellular solution.

As reported in the literature (7, 22), both cold acclimation and freezing injury decreased the low-high frequency resistance ratio (Figs. 9 and 10). It is believed that this ratio is equivalent to the extracellular resistance divided by the intracellular resistance. The decrease with cold acclimation is largely related to the intracellular resistance increase because cold acclimation did not significantly change the extracellular resistance. The magnitude of the ratio measures how effectively the plasma membrane is acting as a barrier to diffusion between the extracellular and intracellular regions. Because freezing to −5.6°C and −8°C produced different ratios (Fig. 9), there are degrees of tissue or membrane injury.

The time constant believed to equal the product of membrane resistance and capacitance was not affected by cold acclimation (Fig. 4). Thus, membrane resistance and capacitance were not changed or were not changed sufficiently to affect the time constant. Cold acclimation had no significant effect on the membrane capacitance of rye protoplasts (12). But the time constant increased with increasing temperature at nonfreezing temperatures (Table II). This is consistent with an expected membrane permeability increase with increasing temperature (10). Because NA stems were injured by freezing to −5.6°C and −8°C, it was not considered valid to calculate a time constant for NA stems at these temperatures. However ACCL stems were not injured at −5.6°C and −8°C and therefore time constants were calculated. Interestingly, the temperature dependence at freezing temperatures for the time constant (Table I) was con-

sistent with the notion that membrane permeability increased with decreasing temperature. In agreement with this, Olien and Lester (15) reported that sugars are released into the extracellular region of frozen but noninjured plants. Also, over certain temperature ranges, permeability has been observed to increase with decreasing temperature in phospholipid vesicles (2) and in dog red cells (17).

In conclusion, at nonfreezing temperatures cold acclimation increased the resistance of the intracellular solution but not the resistance of the extracellular solution. At freezing temperatures the resistance of both the intracellular and extracellular solution was higher for ACCL stems than for NA stems. Therefore, during freezing the development of extracellular electrical transients and the distribution of these transients across the cell membrane could be different for ACCL and NA plants. The ratio of extracellular resistance to intracellular resistance is decreased by both freezing injury and cold acclimation. The resistance ratio decrease occurs while stems are frozen and following freezing and thawing.

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